



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁴ : C07G 17/00, A61K 39/00 C12N 5/00, C12P 21/00		A1	(11) International Publication Number: WO 89/ 07601 (43) International Publication Date: 24 August 1989 (24.08.89)
(21) International Application Number: PCT/GB89/00138 (22) International Filing Date: 15 February 1989 (15.02.89) (31) Priority Application Numbers: 8803756 8803757 (32) Priority Dates: 18 February 1988 (18.02.88) 18 February 1988 (18.02.88) (33) Priority Country: GB (71)(72) Applicant and Inventor: TAN, Kim, Sze [MY/GB]; 52 Farnham Road, Guildford, Surrey GU2 5PE (GB). (74) Agent: GILL JENNINGS & EVERY; 53/64 Chancery Lane, London WC2A 1HN (GB). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent),			US. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: ANTIGENS AGAINST AUTOIMMUNE DISEASES			
(57) Abstract			
<p>An antigenic determinant against a species-related autoantibody, in purified form can be used in the treatment of autoimmune diseases. A hybrid cell comprising a heteromyeloma cell fused to a species-related somatic cell, which is capable of secreting complete cell products of the somatic cell can be used, inter alia, to produce the novel pure determinants.</p>			

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ANTIGENS AGAINST AUTOIMMUNE DISEASESField of the Invention

This invention relates to antigenic determinants for use in the prevention and treatment of autoimmune diseases, and also to hybrid cells which code for, express and secrete biological cell and membrane products, their production and use.

Background of the Invention

Autoantibodies which are produced by B lymphocytes are involved in the pathogenesis of a number of autoimmune diseases, such as thyroiditis or diabetes, which involve organs such as the thyroid, pancreatic islets or adrenal glands. Some autoantibodies bind cell membrane receptors (or antigens) and trigger an immune response which leads to destruction of the cell or antigen. What triggers the autoimmune response is not known. Autoantibodies can also bind antigens, causing stimulation or blockage of biological processes. The circulating levels of autoantibodies in the body may be low, but such autoantibodies are highly potent and have to be neutralised to prevent damage to organs and cells.

Current evidence suggests that Type 1 diabetes mellitus is a chronic autoimmune disease involving two types of islet cell antibodies: (i) islet cell cytoplasmic antibodies (ICCA); (ii) islet cell surface antibodies (ICSA). The ICSA are believed to be central to the initial destruction of the beta cells, whereas ICCA is secondary to the damage. ICSA have been detected in 40-67% of recent-onset diabetics and are present in susceptible individuals long before clinical onset. As such, they may be used as an early predictor of diabetes and as a marker of the silent, ongoing beta cell damage in prediabetic individuals.

Various approaches have been adopted to neutralise autoantibodies, including the use of immunosuppressants,

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e.g. cyclosporin, and of mouse monoclonal anti-idiotypic antibody neutralisation techniques, but neither has been very successful. Immunosuppressants are too non-specific and will attack all B and T cells, while the use of mouse
5 monoclonal anti-idiotypic antibodies often results in the production of human anti-mouse antibodies in patients, considerably reducing the effectiveness of treatment.

Desired biological products can be produced by growing bacterial or yeast cells whose DNA has been
10 modified, by recombinant technology, to express the products. Current genetic transfer/fusion techniques provide cells which are capable of secreting desired polypeptides. However, a desired product containing an amino-acid sequence and an additional moiety such as a
15 carbohydrate, lipid or glycolipid cannot be secreted. The additional moiety must be added to the amino-acid sequence of the product at a later stage in product synthesis. Examples of biological products having such additional moieties are glycoproteins, lipoproteins and
20 glycolipids. Moieties such as lipids or carbohydrates are important in the synthesis of biological products; their presence may be crucial in determining the stability and the biological/immunological activity of the final product.

25 It is known to produce immortal hybrid cells by transfection/fusion of oncogenes or myeloma lines with somatic cells, usually of the same species. The use of such hybrid cells to produce a desired product carries high risk that cancer products will also be produced.
30 Contaminating cancer products could induce cancer growth in individuals that are treated with the product.

An object behind the present invention is to produce biological products comprising amino-acid sequences combined with an additional moiety, preferably in the
35 absence of oncogenic products.

Summary of the Invention

The present invention provides antigenic determinants, i.e. antigens or antigenic fragments, which are specific for autoantibodies and which can be used to neutralise circulating antibodies and to target the B lymphocytes producing the autoantibodies.

The present invention provides also a hybrid cell line which is the product of cell fusion between a heteromyeloma cell and a species-related somatic cell. The hybrid cells are capable of secreting complete cell and membrane products of the somatic cell. The heteromyeloma cells have undergone membrane alteration so that they are species-related to the somatic cells, but have been derived from myeloma cells which are not species-related to the somatic cells.

Detailed Description of the Invention

Antigenic determinants containing the antigenic sequences specific for autoantibody binding sites can be identified by standard gene-sequencing techniques. Once the amino-acid sequence is known, the peptide fragments for the antigen sequence may be synthetically produced. Synthetic production, e.g. by recombinant technology, may be the preferred route for antigenic determinants that are composed of amino-acids only. However, for antigenic determinants which are glycoproteins, lipoproteins or glycolipids, synthesis is less easy. The preferred route to produce such antigenic determinants is from hybrid cell lines of the invention; they are described in more detail below.

Enzyme digestion or chemical treatment of the antigens can be carried out to produce individual antigenic fragments which are able to bind specifically to the autoantibodies, so causing a neutralising effect. An antigenic fragment inevitably has lower molecular weight than the corresponding antigen and may therefore

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be less immunogenic than whole antigens when administered to patients in vivo.

An antigenic determinant of the invention may have as its sole function the blocking of an autoantibody.

- 5 The determinant may also have other functions, and any such other function which is undesirable can be blocked by using a complementary antigen binder.

Antigenic determinants of the invention are essentially free of naturally-associated material. They
10 may be purified by standard techniques. Examples of such techniques are affinity purification, HPLC and electrophoresis.

A determinant of the invention may be incorporated into a composition which can be used to treat patients
15 having autoimmune diseases.

Long-term treatment of autoimmune diseases may involve not only the neutralisation but also the destruction of the B lymphocytes which secrete autoantibodies. Antigenic determinants of the invention
20 can be tagged with radioactive tracers such as iodine or cytotoxic drugs which can then be used to target and destroy autoantibody-secreting B lymphocytes.

Tagging may be of particular value in the prevention or prophylaxis of diabetes, where it is believed that
25 autoantibodies can be detected 5 to 7 years before the patient becomes insulin-dependent; there may therefore be adequate time for individuals to be treated with neutralising agents such as antigenic determinants specific for the autoantibody, up to the stage when 50%
30 (or more) of the pancreas has been destroyed.

Antigenic determinants may also be used to screen biological samples from individuals before an autoimmune disease is manifested. For screening, the antigenic determinants may be incorporated into diagnostic kits.

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Hybrid cells of the present invention can be used to produce desired biological products that are expressed by the somatic cell. It is an advantage of the invention that, if the myeloma cells are of, say, animal origin, no human oncogenic products are secreted in addition to the desired product when the hybrid cell products are used in humans. It is preferred that the hybrid cells are the product of cell fusion between human heteromyeloma cells and human somatic cells.

Standard gene cloning or fusion technologies can be used to produce a human or other heteromyeloma cell line which is derived from, say, another animal myeloma cell line. Standard techniques can be used to fuse cells from the heteromyeloma cell line with cells from a line of selected human somatic cells such as thyroid cells, to produce a hybrid cell line. All the genes of the thyroid cell are incorporated into the hybrid and expressed, and cell products are secreted.

A hybrid human heteromyeloma/thyroid cell line has been deposited on 18th February 1988 at the European Collection of Animal Cell Cultures, Porton Down, England. The accession number is ECACC 88021801. The deposited hybrid cell line secretes human thyroid cell and membrane products such as TSH (thyroid-stimulating hormone) receptor, thyroglobulin and thyroid peroxidase enzyme. There is minimal secretion of human oncogenic products.

Similar techniques to those outlined above can be used to produce hybrid cell lines derived from human somatic cells which are, for example, pituitary cells, pancreatic endocrine and exocrine cells, adrenal medulla and cortex cells, blood cells, liver cells, lung cells, brain cells, bone marrow cells, gut cells, placental cells, ovary cells and testicular cells. The hybrid cells can be cultured to express and secrete products expressed by the somatic cell; provided that the hybrid

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cells retain the appropriate genes, the products will continue to be secreted.

Insulin and other pancreatic hormones and enzymes can be produced from hybrid-containing pancreatic cells, while LH (luteinising hormone), FSH (follicle-stimulating hormone), GH (growth hormone), prolactin and TSH can be produced from hybrid-containing pituitary cells. LH and FSH are recognised to enhance the success rate of in vitro fertilisation procedures. Other products of interest are erythropoietin and TPA (tissue plasminogen activator).

By the procedure of the following illustrative Example, islet cell membrane antigens (ICMA) have been purified from cell lines that express the antigen and have demonstrated binding of the ICMA with human islet cell autoantibodies (ICA). The ICMA can be digested by a variety of enzymes or chemical treatment, and purified fragments tested for antigenic bonding with ICA. ("Guildhay" refers to Guildhay Antisera Ltd., 6 Riverside Business Centre, Walnut Tree Close, Guildford, Surrey, England).

Example

An anti-anti-idiotypic mouse monoclonal antibody (Moyle et al, 1988 Diabetes 37, 206; Moyle et al, 1988 Biochem. Soc. Trans., in press) was used for the purification of the ICMA. 5 mg purified monoclonal antibodies were coupled on to 1 g activated beads (Guildhay). The column was washed 3 times with phosphate-buffered saline (PBS), and a solubilised islet cell membrane preparation, passed through a 0.2 μ m filter (Millipore), was applied to the column. The column was then washed with PBS until there was no protein in the eluate. Bound ICMA was eluted at 4°C, and fractions containing ICMA were pooled and dialysed against PBS.

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The purified ICMA was characterised in the following ways:

(a) Nitrocellulose blotting: Purified ICMA was spotted on to nitrocellulose membranes, incubated with ICSA-positive diabetic sera and anti-anti-idiotypic MAb. Staining was visualised by coomasie blue.

(b) Enzyme-linked immunosorbent assay (ELISA): Purified ICMA was coated on to sensitised microtitre wells, incubated with ICSA-positive diabetic sera and anti-anti-idiotypic MAb. Binding was visualised by adding either anti-human-HRP or anti-Mouse-HRP conjugate (Tan et al, Diabetes 1988, 37:204).

(c) Displacement ELISA: The purified ICMA will displace binding of ICSA-positive diabetic sera in the ICSA ELISA kit available from Guildhay.

(d) SDS-gel Electrophoresis: By classical SDS polyacrylamide gel electrophoresis, the ICMA appeared as a single band with a molecular weight of 60,000-65,000.

(e) Nature: The ICMA is apparently a glycoprotein with sialic acid terminal groups. Neuraminidase treatment, however, did not affect the binding of the ICMA to ICSA-positive diabetic sera.

Because it is believed that circulating ICSA initiates beta cell damage when bound to ICMA, it is possible to use complementary peptides or chemicals to bind the ICMA and thus prevent ICSA binding. In this way, the beta cells could effectively be prevented from damage. The design of these "protective" peptides or chemical compounds follows from knowledge of the amino-acid sequence of the antigenic site of ICMA.

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CLAIMS

1. An antigenic determinant against a species-related autoantibody, in purified form.
2. A determinant according to claim 1, in which the
5 species is human.
3. A determinant according to claim 1 or claim 2, which is a lipoprotein, glycoprotein or glycolipid.
4. A determinant according to any preceding claim, which is a pancreatic islet cell membrane antigen.
- 10 5. A determinant according to any preceding claim, which is labelled.
6. A determinant according to any preceding claim, which is bound to a cytotoxic drug.
7. A determinant according to any preceding claim, for
15 use in the treatment of an autoimmune disease, in which the determinant is species-related to the autoantibodies which cause the autoimmune disease.
8. A vaccine composition comprising a determinant according to any of claims 1 to 7.
- 20 9. A method of treating an autoimmune disease in a subject, which comprises administering to the subject a determinant according to any of claims 1 to 7.
10. A hybrid cell comprising a heteromyeloma cell fused to a species-related somatic cell, which is capable of
25 secreting complete cell products of the somatic cell.
11. A hybrid cell according to claim 10, in which the somatic cell is a pituitary cell.
12. A hybrid cell according to claim 10, in which the somatic cell is an adrenal medulla or cortex cell.
- 30 13. A hybrid cell according to claim 10, in which the somatic cell is a pancreatic cell.
14. A hybrid cell according to any of claims 10 to 13, in which the heteromyeloma cell and somatic cell are human cells.

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15. A hybrid cell according to claim 14, in which the heteromyeloma cell contains genes from an animal cell.

16. A hybrid cell according to claim 10, having the deposit accession number ECACC 88021801.

5 17. A hybrid cell according to any of claims 10 to 16, which is capable of secreting a lipoprotein, glycoprotein or glycolipid.

18. A method of producing a biological cell and/or membrane product, which comprises culturing a hybrid cell
10 according to any of claims 10 to 17.

19. A method according to claim 19, in which the biological product is a lipoprotein, a glycoprotein or a glycolipid.

20. A method according to claim 19 or claim 20, in which
15 the biological product is a determinant according to any of claims 1 to 6, and which comprises the further step of purifying the determinant.

21. A method according to claim 20, which comprises the additional step of enzymatically digesting or chemically
20 treating the antigenic determinant to produce antigenic fragments.

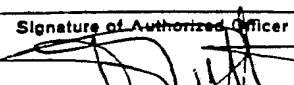
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INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/00138

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC ⁴ : C 07 G 17/00; A 61 K 39/00; C 12 N 5/00; C 12 P 21/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁴	C 12 N; A 61 K; C 12 P	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	GB, A, 2146338 (YEDA R & D CO. LTD) 17 April 1985 see the whole document	1-5,7,8
Y	--	20,21
X	EP, A, 0251107 (MERREL DOW) 7 January 1988 see the whole document	10-19
Y	--	20,21
X	EP, A, 0155676 (MAX PLANCK GESELLSCHAFT) 25 September 1985 see claims 1,2,13	1-3,5,7
X	WO, A, 87/05929 (GENELABS INC.) 8 October 1987 see claims 1,4-6,8-10,12-14 -----	10-19
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"G" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
8th June 1989	05 JUL 1989	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	 P.C.G. VAN DER PUTTEN	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers9....., because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): methods for treatment of the human
or animal body by surgery or therapy,
as well as diagnostic methods

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 8900138
SA 27221

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 28/06/89
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 2146338	17-04-85	CA-A- 1235660	26-04-88
		DE-A- 3433339	28-03-85
		FR-A- 2553100	12-04-85
		JP-A- 60155126	15-08-85
		US-A- 4634590	06-01-87
EP-A- 0251107	07-01-88	AU-A- 7451587	07-01-88
		JP-A- 63003787	08-01-88
EP-A- 0155676	25-09-85	DE-A- 3410049	19-09-85
		DE-A- 3433021	20-03-86
		JP-A- 60224064	08-11-85
		US-A- 4798800	17-01-89
WO-A- 8705929	08-10-87	AU-A- 7281787	20-10-87
		EP-A- 0262211	06-04-88